Current Concepts in Bronchioloalveolar Carcinoma Biology

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Bronchioloalveolar carcinoma (BAC) has a unique clinical and radiological presentation and a different response to systemic treatments compared with conventional lung adenocarcinoma. Although tobacco-related, BAC is found disproportionately in never-smokers, women, and Japanese patients. The molecular basis for these predispositions is unknown. The WHO defines BAC as a subtype of adenocarcinoma with growth along the alveolar septa and without evidence of stromal, vascular, or pleural invasion. Although only ~4% of lung cancers meet this definition, up to 20% of lung cancers comprise a heterogeneous group of tumors with BAC histology mixed with a varying population of invasive cells, ranging from predominant BAC histology with a small focus of invasion, to invasive adenocarcinoma with an isolated group of cells with BAC features at the periphery (2–4). Tumors with BAC histology are associated with improved survival rates compared with pure adenocarcinoma. A subset of BAC, mucinous BAC, has poorer outcomes than nonmucinous BAC and accounts for ~20% of BACs. In contrast to nonmucinous BACs, which most commonly present as small peripheral nodules, mucinous BACs frequently masquerade as pneumonia often resulting in a delay in diagnosis (5).

The proportion of tumors with BAC features may have increased in incidence over the past 50 years (6). A population-based study using the Surveillance, Epidemiology, and End Results database found that BAC accounted for only 4% of non–small cell lung carcinomas (NSCLC), a percentage that did not significantly change during the last 25 years (2). However, several single institution series report that BAC accounts for >20% of NSCLC, with some series reporting numbers as high as 40% (6–8). This discrepancy is likely due to the inclusion of BAC tumors mixed with adenocarcinoma in most single institution series, which would have been classified as adenocarcinoma in the Surveillance, Epidemiology, and End Results registry. Moreover, most institutional series of BAC have studied patients with surgically resected lung cancer. BAC seems to be more common in early stage lung cancer, and there may be misclassification of BAC as adenocarcinoma in patients with surgically resected lung cancer because these patients typically are diagnosed by cytologic means, which has poor sensitivity for BAC (9). Because two-thirds of patients with NSCLC present with metastatic or surgically unresectable disease, a large number of patients with advanced stage lung adenocarcinoma in the Surveillance, Epidemiology, and End Results registry may in fact have BAC histology. The reasons for the apparent increase in incidence in BAC reported by several groups may include increased detection of small lung cancers with increased thoracic imaging, increased reporting of BAC features by pathologists, or an actual increase in incidence due to a viral etiology or other environmental factor (10).

Multistep Model of Lung Cancer Development: BAC as Carcinoma in situ

The concept of oncogenesis as a multistep process during which genetic mutations are sequentially accumulated resulting in the development of an invasive phenotype has been well established in a number of cancers, including colon and breast cancers (11, 12). A growing body of literature supports a similar model in lung adenocarcinoma development. BAC and atypical adenomatous hyperplasia (AAH), a premalignant lesion thought to be a precursor to BAC, are frequently found adjacent to areas of invasive adenocarcinoma (13–15). AAH is a small uniform cluster of atypical cells spreading in single rows along the alveolar septa (see Fig. 1; ref. 14). BAC is a carcinoma in situ, because according to definition it lacks vascular, lymphatic, or mesenchymal invasion. Histologically, a spectrum of bronchioloalveolar atypia and invasion could be found (13). Tumors characterized as pure BAC show more frequent atypia and growth along the alveolar septa with maintenance of the elastic alveolar architecture (Fig. 1). As tumors begin to have invasive potential, central desmoplastic reactions and elastic framework destruction are found. Vascular and lymphatic channel and basement membrane invasion are the next features to appear, along with more diffuse tumor growth. The signals that cause atypical bronchioloalveolar cells to either transform rapidly into invasive adenocarcinoma or to maintain the predominantly noninvasive, but often diffusely infiltrating, growth pattern that is seen in BAC are currently unknown. The clinical and histologic heterogeneity of BAC suggest that there are multiple pathways of genetic changes that can result in the progression from atypia to invasive adenocarcinoma. The occasional finding of AAH, BAC, and adenocarcinoma in one lung cancer sample suggests that even within a single tumor, heterogeneous molecular events occur during lung neoplastic development, possibly originating from a clonal population of lung cancer stem cells.

Bronchioloalveolar Stem Cells and Lung Adenocarcinoma

A mouse model of lung adenocarcinoma, reported by Jackson et al., using a conditionally expressed K-ras-activated mutation, showed the progression of clusters of AAH to what the investigators term, epithelial hyperplasia and adenomas,
which bear a striking resemblance to BAC and to invasive adenocarcinoma. By developing a mouse model that allows for mutated K-ras activation upon infection with an adenovirus construct, the investigators were able to time the initiation of tumorigenesis and reliably obtain tumors at various time points along the transformation to adenocarcinoma. These results validate the multistep model of lung cancer development that is initiated by a driving mutation, in this case, K-ras (16). Recently, the same group described bronchioalveolar stem cells, a population of cells found at the bronchioloalveolar junction capable of generating both terminal bronchiolar epithelium, or Clara cells, and alveolar type II pneumocytes. K-ras activation in a similar mouse model led to the expansion of bronchioalveolar stem cells and differentiation towards an alveolar lineage. Bronchioalveolar stem cell proliferation was found alongside AAH and adenomas, the lesions resembling BAC in mice. Proliferation and adenoma formation was augmented by the induction of airway injury with naphthalene before K-ras activation (17). These data suggest that lung tumorigenesis is initiated by the transformation of bronchioalveolar stem cells, and that the differentiation of this transformed stem cell population depends on a dominant mutation or group of mutations that establish the tumor cell milieu. An investigation into the signals supporting BAC predominant differentiation is under way.

**Tumor Markers in BAC**

Molecular genetic studies examining oncogenes commonly found in NSCLC have supported the model of BAC tumor progression (Fig. 2). Aberrant expression of p53, Ki67, K-ras, Survivin, Her-2 Neu, FHIT, and other markers are accumulated along the spectrum of BAC progression to invasive cancer (Table 1; refs. 15, 18–21).

The tumor suppressor gene p53 is significantly altered in a majority of lung adenocarcinomas and is strongly associated with tobacco exposure (22). p53 was overexpressed in only 8% of AAH and 4% to 16% of pure BAC tumors compared with 35% to 53% of adenocarcinomas (15, 19). Tumors with mixed BAC histology had intermediate levels of expression. Terasaki et al. showed that BAC with <5 mm of invasion had significantly less p53 expression than did tumors with >5 mm of invasion (23). Other investigators have analyzed the frequency of p53 mutations and found similar proportions of
AAH, BAC, and adenocarcinoma with the mutations (24). Meanwhile, no mucinous BAC tumor had p53 mutations (25). Overexpression of p53 seems to be an independent predictor of decreased long-term survival in BAC tumors and stage 1 adenocarcinoma. Survival is further decreased when truncating or structural mutations in the p53 gene are present (26). These results suggest that p53 mutations play an important role in the malignant transformation of BAC.

Although the proto-oncogene K-ras is frequently overexpressed in conventional lung adenocarcinoma, it is infrequent in nonmucinous BAC. In one series, all 10 mucinous BAC samples showed K-ras overexpression with mutations at codon 12, compared with just 23% of nonmucinous BAC (27). Nakanishi et al. showed that the inhibitor of apoptosis protein survivin was expressed in all 40 BAC tumors sampled, compared with just 9% of low-grade AAH (18). This implies a resistance to apoptotic signaling in BAC compared with AAH.

Investigators have similarly analyzed the expression of Ki-67, a nuclear antigen expressed throughout the cell cycle and correlating well with mitotic activity in tumors. Kitamura et al. found increasing Ki-67 expression with increasing grades of AAH (28), and Terasaki et al. described an increasing Ki-67 labeling index when pure BAC, tumors with BAC and increasing proportions of invasion, and pure adenocarcinoma were analyzed. Ki-67 tends to be differentially overexpressed in the marginal areas of mixed BAC tumors compared with the invasive tumor center. In contrast, Ki-67 expression becomes more diffuse in predominantly invasive cancer (23). Laminin-5, an extracellular matrix protein, is strongly expressed in the leading edge of a number of cancers. In one study, no pure BAC tumors expressed laminin-5, but there was a higher proportion of laminin-5 overexpression in mixed BAC tumors with an increasing proportion of invasive cancer, and 38% of adenocarcinoma expressed laminin-5 (23). Awaya et al. recently documented the loss of membranous expression of the cell-cell adhesion molecules E-cadherin and β-catenin with histologic progression from AAH to BAC to adenocarcinoma. Their findings suggest that membranous β-catenin expression (96% of AAH, 70% of pure BAC, and 48% of invasive predominant mixed BAC) might be lost at an earlier stage in lung adenocarcinoma development than the loss of E-cadherin expression (100% of AAH expressed E-cadherin, compared with 93% of pure BAC, and 13% of invasive predominant mixed-BAC). Moreover, loss of E-cadherin correlated with the presence of lymph node metastases and tumor stage. Loss of β-catenin was not associated with lymph node metastases or tumor stage, but was associated with loss of E-cadherin, again implying that the loss of β-catenin expression is an earlier event in cancer development than loss of E-cadherin expression. Also, the elastic fiber network was preserved in BAC tumors even after loss of E-cadherin and β-catenin, suggesting that loss of E-cadherin and β-catenin occurs before architectural destruction by tumor cell invasion (29).

Sarkaria et al. showed increased expression of squamous cell carcinoma–related oncogene in pure BAC, and in two groups of mixed BAC tumors with increasing amounts of invasion. Interestingly, they found low levels of squamous cell carcinoma–related oncogene expression in adjacent normal lung tissue that correlated with a significantly worse survival. This data suggests either preatypia changes in host cells resulting in predisposition to malignant transformation, or possibly host-tumor interactions leading to oncogenesis (30). More studies on the effect of host characteristics such as gender, smoking status, and ethnicity on BAC and adenocarcinoma development by analysis of normal lung tissue in patients with BAC are needed.

**Chromosomal Abnormalities in BAC**

Studies investigating chromosomal alterations by way of microsatellite marker analysis have provided additional insight into the genetic changes in BAC. During progression from AAH through invasive predominant adenocarcinoma, there is progressive fractional allelic loss as well as heterogeneity of allelic variation.
loss within the tumor sample (Fig. 2; refs. 31, 32). Interestingly, Dacic et al. found decreased fractional allelic loss in adenocarcinomas with lymph node metastases compared with those without lymph node metastases, and an even greater decrease in fractional allelic loss within the lymph node metastasis when compared with the primary tumor (31). This finding suggests that there may be significant mutational heterogeneity early in invasive cancer until there is clonal selection of cells capable of metastasis. Allelic loss of 9p is commonly found in AAH and in early BAC, indicating that a gene in that region may be responsible for early changes associated with BAC formation (32, 33). Cyclin-dependent kinase inhibitor 2a (p16), a tumor suppressor gene, is found in this region and has been an area of investigation. Loss of heterozygosity of 8q and 17p, containing Myc and p53, respectively, are found more frequently in BAC compared with AAH, and still other allelic losses (1p, 3p, 7q, 10, 17p, 18q, and 21) are found more commonly in adenocarcinoma compared with BAC. Genes such as FHIT, MycL1, VHL, and DCC are found in these chromosomal regions, potentially representing genes related to the invasive properties of adenocarcinoma (31, 32). The FHIT tumor suppressor gene located on 3p14 is important in a number of malignancies, and loss of expression by allelic loss or promoter hypermethylation is common and predicts poor prognosis in lung cancer (34). MycL1 is a nuclear oncogene located on 1p34 that controls cell proliferation and differentiation and might be associated with poor prognosis in lung cancer (35). Inactivation of VHL, a tumor-suppressor gene located on 3p25-26 responsible for the von Hippel-Lindau hereditary cancer syndrome, is associated with lung cancer development possibly through the activation of angiogenesis (36). Expression of DCC, a tumor suppressor gene located on 18q21, is lost in a number of cancers. A member of the family of dependence receptors, DCC is involved in the induction of apoptosis, and loss of DCC expression plays a role in colorectal adenocarcinoma development (37). Although these studies provide some support for the progression of BAC to adenocarcinoma, they also point to the heterogeneity and complexity of the chromosomal alterations involved in lung cancer. In one study, 94% of adenocarcinomas had regions containing different chromosomal alterations when various histologic regions were laser capture microdissected and analyzed (32). The frequent clinical finding of AAH, BAC, and adenocarcinoma in the same lung cancer sample suggests that molecular events often occur heterogeneously, perhaps beginning with mutations in the bronchoalveolar stem cells, and that there might be multiple molecular pathways of progression to adenocarcinoma. Furthermore, the genetic and chromosomal differences in BAC that confer a diffuse infiltrating phenotype, a multifocal phenotype, or a small nodular phenotype are unknown and warrant further investigation.

### Table 1. Relative frequencies of tumor marker expression among AAH, BAC, and lung adenocarcinoma tumors

<table>
<thead>
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<th>Marker</th>
<th>AAH (%)</th>
<th>Pure BAC (%)</th>
<th>Mixed BAC (%)</th>
<th>Mucinous BAC (%)</th>
<th>Adenocarcinoma (%)</th>
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<tr>
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<td></td>
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<td>35</td>
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<tr>
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### Epidermal Growth Factor Receptor Mutations in BAC

Patients with metastatic BAC have disproportionately responded to the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI), gefitinib and erlotinib. In clinical trials evaluating the use of gefitinib in advanced chemotherapy-refractory NSCLC, the factors that predicted response to gefitinib after multivariate analysis were female gender, never-smoking status, Japanese ethnicity, and BAC histology (38). In fact, 38% of patients with BAC histology responded to gefitinib compared with 8% of all other adenocarcinomas (39, 40). Curiously, EGFR expression by immunostaining does not predict a response to gefitinib (41). Investigators have found that mutations near the ATP binding cleft of the tyrosine kinase domain of EGFR correlate with response to EGFR-TKIs. Amino acid substitutions or deletions in exons 18, 19, and 21 were found in 93% of responders to gefitinib, compared with 0% to 13% of nonresponders (42). Interestingly, the frequency of EGFR mutations among Japanese patients with any lung cancer is 39% (43), and 48% among Taiwanese patients (44), compared with 3% to 9% among patients from the U.S. (45). There has been no epidemiologic study investigating the prevalence of EGFR mutations among ethnic Asians born outside of Asia and whether EGFR mutations decrease in ethnic Asians living in Western countries for several generations. Moreover, the genetic or environmental basis for this ethnic predominance of somatic EGFR mutations is unknown.

In a study of EGFR mutations in 860 lung cancers, Marchetti et al. showed that none of the mucinous variant BAC tumors had EGFR mutations compared with 32% of nonmucinous tumors and 57% of nonsmokers with BAC histology. Interestingly, K-ras mutations were mutually exclusive of EGFR mutations. Nonmucinous (14%) and mucinous (76%) BAC tumors had K-ras mutations, but no tumor that carried an EGFR mutation had K-ras mutations (46). Not surprisingly,

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**K-ras mutations** are more common in EGFR-TKI nonresponders (47). The reasons for this striking finding is unclear. K-ras is strongly associated with smoking, whereas EGFR mutations are strongly associated with nonsmoking status. It is possible that the association between EGFR and K-ras mutations is not independent, but is confounded by smoking status. Alternatively, EGFR mutations and K-ras mutations might each independently drive tumorigenesis in functionally similar ways, making it unlikely for tumors to have both EGFR and K-ras mutations. Prospective trials evaluating response to EGFR-TKIs including EGFR and K-ras sequencing are under way to help shed light on this intriguing finding.

As previously mentioned, loss of expression of the adhesion molecule, E-cadherin, plays an important role in lung cancer progression. EGFR interacts with E-cadherin, and expression of E-cadherin predicts response to EGFR inhibitors. Recently, Witta et al. showed that the restoration of E-cadherin expression led to growth inhibition and apoptosis after gefitinib treatment in gefitinib-resistant lung cancer cell lines. This report underscores the important interactions between E-cadherin and the epidermal growth factor pathway in BAC (48).

It is unclear why a gain of function EGFR mutation is associated with BAC histology and is infrequently found in less differentiated adenocarcinomas. It is possible that nonsmokers, women, and Asians are predisposed to developing EGFR mutations and are independently predisposed to developing BAC histology through other pathways, such that BAC histology confounds a true association between smoking status, gender, and ethnicity and EGFR mutation status. The development of an animal model of EGFR gain of function mutation is important in understanding the role of EGFR mutations in the development of BAC and lung adenocarcinoma.

**Signaling**

**EGFR pathways**

Two recent investigations on the expression of downstream genes in the EGFR pathway have shown that the EGFR mutations found in BAC activate antiapoptotic pathways rather than proliferative pathways. After ligand binding to EGFR, the receptor dimerizes, either by homodimerization orimerization to nearby ERB family receptors, most commonly ERBB2 (HER-2). This leads to phosphorylation at specific tyrosine kinases in the intracellular domain of the receptor, activating downstream signaling pathways. The two dominant pathways are the phosphatidylinositol 3’-kinase/Akt pathway, involved predominantly in the control of apoptosis, and the Ras mitogen-activated protein kinase pathway, initiated by Ras activation leading to ERK1- and ERK2-mediated regulation of cell proliferation signals (Fig. 3; ref. 49). Cappuzzo et al. showed that Akt phosphorylation, which activates Akt, in lung cancers, was associated with BAC histology, never-smoker status, and female gender. Mitogen-activated protein kinase phosphorylation showed no correlation with those factors. Sixty percent of Akt phosphorylation–positive tumors showed a response to gefitinib (50). Sordella et al. showed that EGFR mutations in cell lines resulted in selective Akt pathway activation leading to antiapoptotic signals which are inhibited in the presence of gefitinib. These cell lines showed marked resistance to the chemotherapeutic agents cisplatin and doxorubicin; however, small interfering RNA targeting the mutated EGFR resulted in rapid apoptosis (51). This finding suggests that the response of EGFR-mutated tumors to EGFR-TKIs results from the inhibition of Akt-mediated antiapoptotic pathways to which the tumor has become exquisitely sensitive.

**Wnt pathways**

Understanding the signaling pathways related to EGFR that are active in BAC development and progression is critical and may shed light on other pathways that may prove to be important in BAC (Fig. 3). For example, investigators have found that overexpression of genes in the Wnt/β-catenin pathway of oncogenesis, specifically Wnt-1, Wnt-3A, Wnt-5, and β-catenin result in EGFR up-regulation and activation (52, 53). These results suggest that the Wnt pathway may
play an important upstream role in EGFR-sensitive tumor development. In fact, preliminary data from our group has shown that Wnt-1 and Wnt-2 are significantly overexpressed in a BAC cell line and in several human BAC tumors (54, 55). Furthermore, Wnt-2-specific small interfering RNA and antibody treatments in a Wnt-2-expressing BAC cell line, A549, induced apoptosis, suggesting that inhibition of Wnt-2 expression or function may have potential therapeutic use in the treatment of human BAC (54, 55). Although membranous β-catenin expression is commonly lost early in the transformation from BAC to adenocarcinoma, it is unclear if cytosolic or nuclear β-catenin expression is similarly lost. Loss of cytosolic β-catenin expression suggests that Wnt overexpression may be especially important early in the development of adenocarcinoma. Our group has speculated that aberrant Wnt pathway expression may plan an important role in the transformation of cancer stem cells due to the important role of the Wnt pathway in developmental biology (46). Alternatively, Wnt pathway expression may exert its downstream effects in BAC and lung adenocarcinoma via noncanonical pathways, which do not involve β-catenin as an intermediary signal.

Neutrophil infiltration in BAC

Finally, there is evidence that the degree of neutrophil infiltration in BAC is associated with a poorer clinical outcome, especially in mucinous BAC. BAC cells have been shown to drive neutrophil recruitment and proliferation by interleukin-8 and granulocyte macrophage colony-stimulating factor production, whereas neutrophil-derived chemokines drive tumor proliferation and local infiltration (56, 57). Whether neutrophil-associated signaling is essential to mucinous BAC growth or represents a potential target for therapy is unknown.

Current treatment of BAC

The only potentially curative therapy for BAC is surgical resection. Localized BAC is treated like other NSCLCs with lobar lung resection and ipsilateral mediastinal lymphadenectomy. Most BACs are amenable to lobectomy, although bilobectomy and pneumonectomy are sometimes required for complete resection of diffuse or multifocal BAC. Completely resected BAC is associated with a 48% to 69% 5-year survival. Of patients who recur, 76% to 95% initially recur locally, a rate higher than other subtypes of NSCLC (5, 58). Survival after surgical resection is associated with early stage, nonmucinous subtype, and absence of vascular or lymphatic invasion (8, 59). As previously discussed, it is controversial whether a higher proportion of BAC histology predicts worse outcomes. Of patients who recur, 76% to 95% initially recur locally, a rate higher than other subtypes of NSCLC (5, 58). Survival after surgical resection is associated with early stage, nonmucinous subtype, and absence of vascular or lymphatic invasion (8, 59). As previously discussed, it is controversial whether a higher proportion of BAC histology predicts worse outcomes.

Future directions in BAC

Understanding the genetic changes that occur in BAC between a small preinvasive tumor and locally advanced or metastatic BAC is paramount. The development of an animal model of BAC based on an EGFR mutation seems important to answer this question. Work on the mutations and tumor milieu that determine the direction and pace of lung cancer stem cell differentiation towards BAC is needed. For example, smoking exposure or lack of estrogen might create an environment for lung cancer stem cells that favors the differentiation of stem cells towards a more aggressive form of lung cancer. Host-tumor interactions and somatic EGFR mutations in normal lung tissue in populations disproportionately affected by BAC may play an important role in BAC development. Although EGFR has emerged as a signal that seems to be associated with these clinical characteristics, other critical signals will likely emerge and represent important targets for therapy for patients with BAC.

Acknowledgments

We thank Kirk Jones M.D. and Anthony Kamezis M.D., Ph.D. for supplying us with the histology images used in this manuscript.
References


